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**Determination and comparison of triacylglycerol composition of tea
(*Camellia sinensis*) oil and extra virgin olive oil using ^1H NMR and UV–
Visible spectroscopy**

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Abstract

The authentication and quality assessment of edible oils remain essential due to increasing cases of adulteration and the strong dependence of oil quality on origin, processing, and storage. Tea seed oil and extra virgin olive oil are particularly valued for their high nutritional and medicinal benefits, yet both contain very low levels of ω -3 triunsaturated fatty acids, making accurate quantification analytically challenging. In this study, we establish a simple and reliable quantitative approach for determining the triacylglycerol (TAG) profiles of tea seed oil and extra virgin olive oil using high-resolution ^1H NMR (800 MHz), supported by UV–Visible spectroscopy. The enhanced magnetic field strength allowed us to resolve the bisallylic proton signals at δ 2.69–2.77 ppm, enabling direct and precise calculation of linoleic (ω -6), linolenic (ω -3), and oleic (ω -9) acyl groups through a modified integration method. The TAG composition of tea oil was determined to be 74.83% (ω -9) monounsaturated, 10.14% (ω -6) diunsaturated, 0.38% (ω -3) triunsaturated, and 14.65% saturated fatty acids. In comparison, extra virgin olive oil contained 81.06% (ω -9) monounsaturated, 4.14% (ω -6) diunsaturated, 0.26% (ω -3) triunsaturated, and 14.54% saturated acyl groups. Both oils are therefore predominantly composed of (ω -9) monounsaturated TAGs. High-resolution ^1H NMR (800 MHz) spectra of both oils resolved all known characteristic signals of edible oils, along with two additional signals. These findings indicate that low concentrations of ω -3 triunsaturated acyl groups can be rapidly and accurately quantified by ^1H NMR, owing to the resolved bisallylic hydrogen signals at δ 2.69–2.77 ppm, which enable the use of a modified equation for TAG calculation. The TAG profiles of tea oil closely match those of extra virgin olive oil observed here and reported in the literature. Furthermore, UV–

Visible absorption spectra measured for six different commercial edible oil samples support these results. Consistent NMR findings, the spectral pattern and intensity of tea oil closely resemble those of high-quality extra virgin olive oil, based on measurements of 1% (v/v) solutions in hexane.

Introduction

Tea and Olive oils are among the most important vegetable oils widely used as favorable food stuff for human nutrition and diet. Many medical researchers have demonstrated the importance of tea and olive oil consumptions due to reach on antioxidant components, anti-aging properties, as a remedy for cardiovascular diseases as well as for bone and nervous system development [1-7]. This creates a unique market dynamic for tea and olive oils being more expensive and sometimes harder to obtain which may present the opportunity for producers and merchants to increase their amount by deliberately adulterating it with cheaper and low graded vegetable oils causing a threat for public health [8-14]. Moreover, the quality of oils often varied in accordance to their origins, manufacturing processes, handling and way of preservation, which makes a need to establish a reliable analytical method for quality assessment and oil authenticity [15-18]. Since majority of the oil components (95-98%) is made up of the triacylglycerols (TAG), the identification and relative quantification of different acyl groups rather than fatty acids by themselves, is one of the important task to determine and control the oil quality [19,20]. Triacylglycerols (TAGs) consists three fatty acids on a glycerol backbone and are very complex due to the variation in chain length, degree of unsaturation and positional distribution between sn-1, sn-2, and sn-3, making each oil type to differ in their quality and application [21-23]. Different methodologies have been applied to detect and quantify TAGs in oil samples, including mass spectrometry, chromatography and NMR spectroscopy. MALDI-TOF/MS and electron ionization tandem mass spectrometry were applied to detect and quantify the TAGs value in Olive and vegetable oil with very similar results to the conventional methods by GC and HPLC [19,20,23].

Nuclear magnetic resonance (NMR) has also become one of the most promising methods to determine oil and fat composition and quality, preferable for its fast, accurate and direct analysis (without sample preparation). Many studies have applied low to medium resolution NMR spectroscopy for determination and quantification of TAGs in several oil types [18, 24-30]. In principle, the area of signals of ^1H NMR spectra is proportionate to the number of hydrogen atoms which allows to quantify the proportions of saturated, monounsaturated, di-unsaturated and tri unsaturated acyl groups in a precise and accurate manner. For example, the ^1H NMR area appearing around δ_{H} 0.83-0.93 ppm is due to the total methyl hydrogen atoms in saturated, oleic and linoleic acyl groups. While the ^1H NMR area appearing around δ_{H} 0.93-1.03 ppm is proportional to the number of methyl hydrogen atoms in triunsaturated acyl group which is important for quantification of triunsaturated proportions. However, oils like tea and olive have small quantity of tri unsaturated acyl group which either merged its signal with the larger portion appearing at δ_{H} 0.83-0.93 ppm or difficult to integrate from the back-ground noise. The absence of the signal at δ_{H} 0.93 ppm resolved by lower than 750 MHz NMR leads many research works to conclude as the oil type has no tri-unsaturated fatty acids. To precisely quantify the proportions of acyl group in tea oil, only few reports have applied higher resolution ^1H NMR with magnetic field of around 750 MHz [17].

In this work, we aimed to establish a quantitative approach for examining the quality of tea seed and olive oil with lower quantities of tri-unsaturated acyl groups by high resolution ^1H NMR spectrometer (800 MHz). The contents of unsaturated fatty acids, namely oleic (ω -9), Linoleic (ω -6), Linolenic (ω -3), and saturated fatty acids (**Figure 1**) were determined according to a novel but simple calculation by resolving the

bisallylic hydrogens at δ_H 2.69-2.77 ppm which are important to use a modified equation to calculate TAG values. Based on our data, the quantitative fatty acid contents of tea seed oil have very similar and comparable to that of extra virgin olive oil and the obtained result is similar to the measured in conventional method reported in literature are quite similar. Such an approach can be applied to other edible oils with lower content of tri-unsaturated fatty acid values (ω -3) to measure the quality of the products in an accurate, fast and quantitative manner.

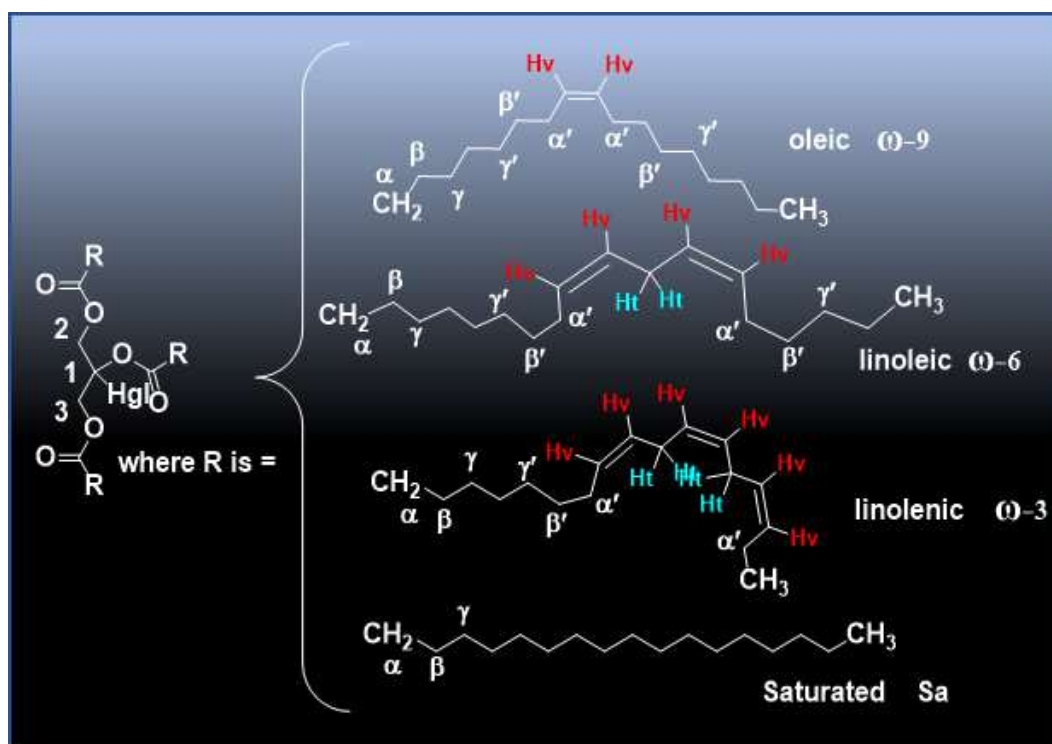


Figure 1: Chemical structure of Triglyceride major component of oils and fats

Experimental

Materials and methos

D-chloroform (purity $\geq 99\%$), Sigma–Aldrich, Germany, was used without further purification for NMR solvents. UV-Visible spectrophotometer, 8453, Agilent, USA, Fourier transform infrared spectrometer (Perkin Elmer Spectrum 100, USA) were used for characterization of oil samples.

Oil samples

Tea seed oil sample was purchased and used for the entire experiment. It was manufactured in Taipei, Taiwan in 2018. Coconut oil, sunflower oil, soybean oil, vegetable oil and extra virgin olive oil have been also purchased in local supermarket in Taipei, Taiwan. All the oil samples were covered in aluminum foil and kept in a refrigerator at room temperature.

Preparation of oils for UV-Visible analysis

Oil samples were prepared as 1% v/v concentration in hexane. 60 μl of each oil samples were taken by micropipette and diluted in 6 ml of hexane solvent. Hexane solvent was used as a blank for UV absorbance measurement.

NMR analysis

^1H NMR analyses were performed on Varian Mercury 400 (400 MHz for ^1H , ^{13}C , 2D COSSY, 2DNOESY, HMBS, HMQC) and 800 MHz for high resolution NMR analysis. Appropriated amount of each oil sample was dissolved in CDCl_3 (500 μl , Sigma-Aldrich, Saint Louis, Missouri, USA) and was placed into a 5-mm diameter ultra-precision NMR sample tubes. The temperature of the sample in the probe was 30 $^{\circ}\text{C}$. The chemical shifts

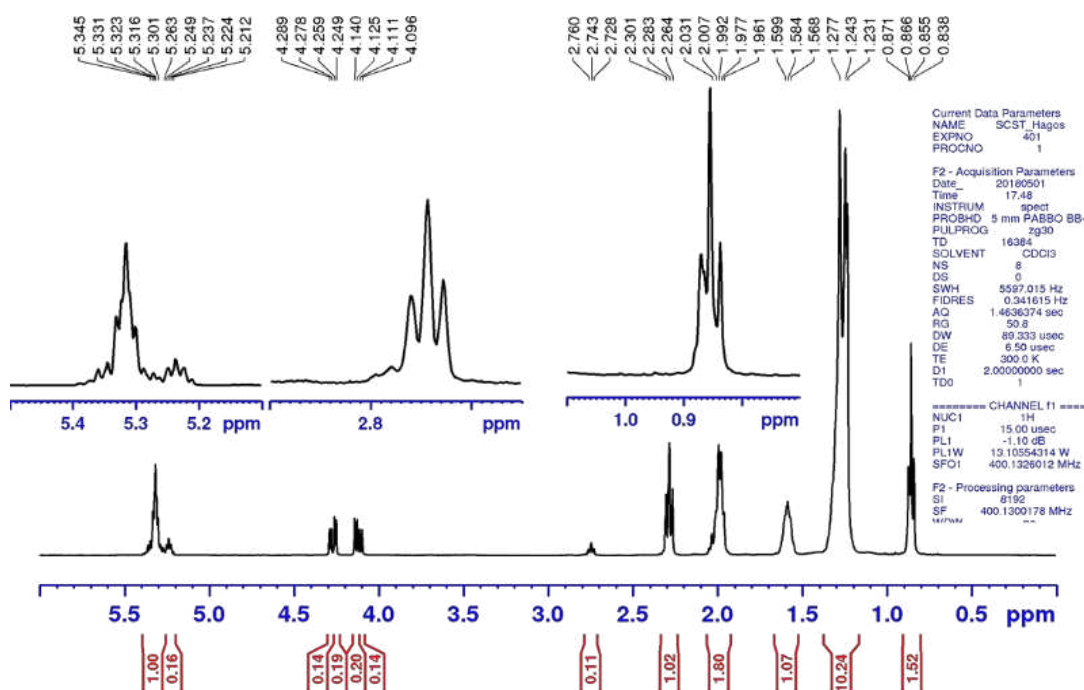
are reported in ppm, using the solvent proton signal as standard. The area of the signals was determined by using the equipment software Topspin 3.5, and the integrations were carried out three times to obtain average values.

Results and discussion

¹H NMR analysis

¹H NMR spectra of tea and olive oils are presented in **Figures 2** and **3** while the integral signal of individual protons is given on **Tables S1** and **S2**. Most oil samples have similar type of ¹H NMR signals differing on the intensity of each proton types. Careful analysis of this area value of ¹H NMR spectral signal difference gives an important information on the type and proportion of acyl groups with very good agreement to the classical analysis methods. General assignment for most of the proton signals are well known in the literature. Here we have revised the signals based on the high-resolution ¹H NMR signal. Signal **a** appearing around δ_H 5.26-5.32 ppm of **Figure 3** is due to the olefinic protons of the different acyl groups and could be used to calculate for total unsaturation amount. Signal **b** appearing at δ_H 5.19-5.24 ppm is due to the single methine proton on the glycerol backbone which often merges with the large signal from olefinic protons. Signal **c** appearing at δ_H 4.25 ppm and **d** at δ_H 4.11 ppm corresponds to the protons of carbon atoms 1 and 3 of the glyceryl group. A new signal **e** appearing around δ_H 3.64 - 3.68 ppm tentatively assigns for the proton on OH group of diglyceride group present in small amount. Signals **f** and **g** are the important signals for directly quantifying the linolenic and linoleic amount which are always appearing as one signal for medium resolution ¹H NMR spectra. This signal **f** appearing around δ_H 2.73-2.78 ppm is due to

the methylenic protons in relation to three double bonds (linolenic acyl groups). While signal **g** appearing around δ_H 2.69-2.74 ppm are due to the methylenic protons in relation to two double bonds (linoleic acyl groups). Proton signals of **h** appears around δ_H 2.24-2.29 ppm is due to methylene hydrogen atoms in α position to the carbonyl group.



bond. Each unsaturated acyl group contains four proton signals appearing at the position **i** which uses to quantify total amount of unsaturation.

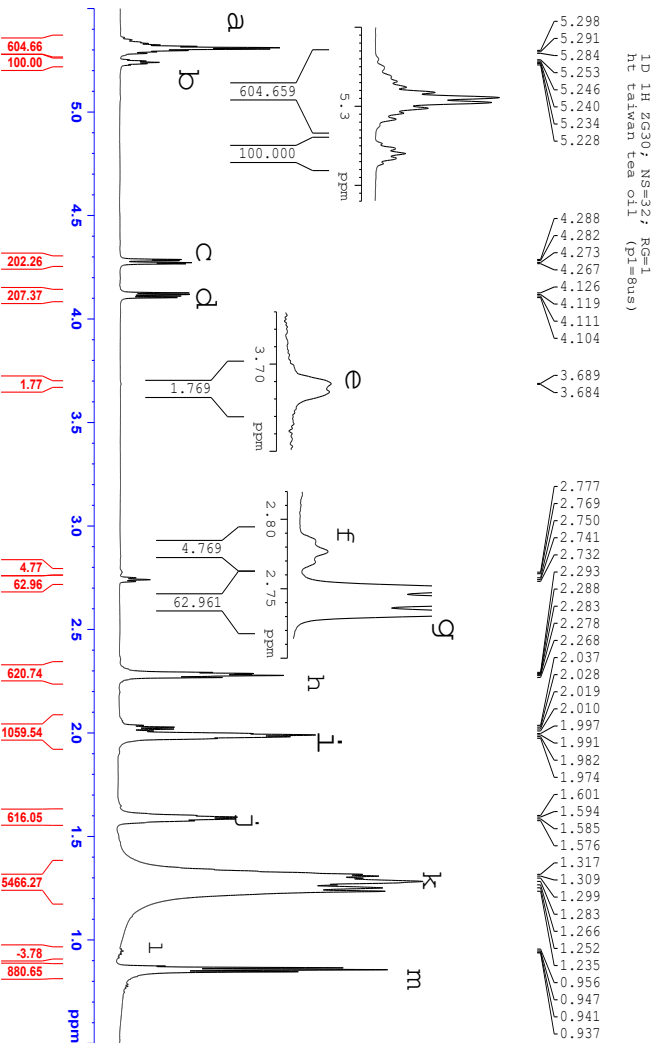


Figure 3a: ¹H NMR of tea oil sample (800 MHz)

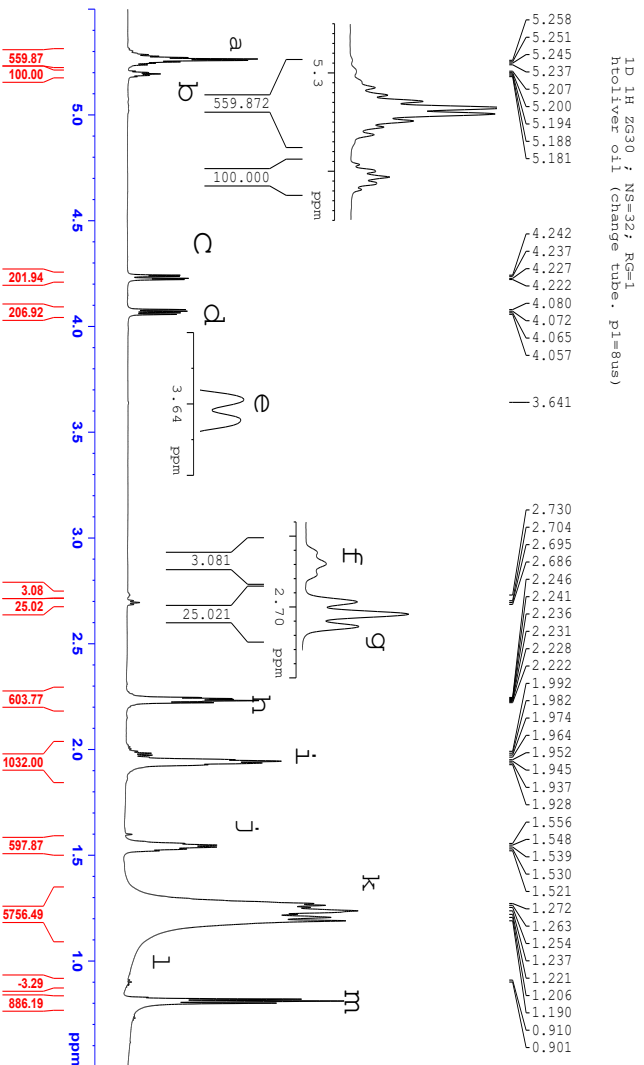


Figure 3b: ¹H NMR of olive oil sample (800 MHz)

Proton signal **j** appearing around δ_H 1.54-1.59 ppm is due to the methylene hydrogen atoms in β position in relation to the carbonyl group. The larger signal **k** is a collective signal due to proton signals in appearing β or further from the olefinic group as well as in position of γ or further from carboxylic groups from the triglyceride molecules. Most literature works using NMR for quantification of triglycerides use signals appearing at **l** and **m** positions to quantify fractions of linolenic and linoleic acyl groups. Signal **l** is due to methyl protons of linolenic acyl group appearing around δ_H 0.89-0.94 ppm due to its proximity to the double bond in position 3. While signal **m** is due to the terminal methyl group protons of saturated and unsaturated acyl groups with double bond in position of 6 or further appearing as triplets between δ_H 0.80 to 0.85 ppm. Even though the proton signal of **m** for both tea and olive oil sample clearly resolves in both 400 MHz and 800 MHz, the important signal **l** used for calculating linolenic amount either disappears or present with large back ground noises **Figures 2** and **3**. This makes us to resolve the proton signals of tea and olive oil samples using high resolution NMR which resolve and separates another important signal of **f** and **g** used for direct quantification of the lower quantity linoleic and linoleic acyl group in an accurate and precise manner.

Table 1: percentage of acyl group type, and simple mathematical formula applied			
Acyl group name	Mathematical formula	Tea oil (%)	Olive oil (%)
Linolenic	$Ln = (f/2h) \times 100$	0.38	0.26
Linoleic	$L = (g/h) \times 100$	10.14	4.14
Total unsaturation	$Unsa = (i/2h) \times 100$	85.35	85.46
Oleic	$O = Unsa - Ln - L$	74.83	81.06
Saturated	$Sa = (1 - Unsa) \times 100$	14.65	14.54

Based on this novel but simple method we have calculated the amount of different acyl groups in tea oil as: Oleic = 74.83%, linoleic = 10.14%, linolenic = 0.38% and saturated = 14.65%, respectively. While the calculated amount of TAG values of extra virgin olive oil is: Oleic O = 81.06%, Linoleic L = 4.14%, Linolenic Ln= 0.26% and saturated S = 14.54%, respectively (**Figure 4** and **Table 1**).

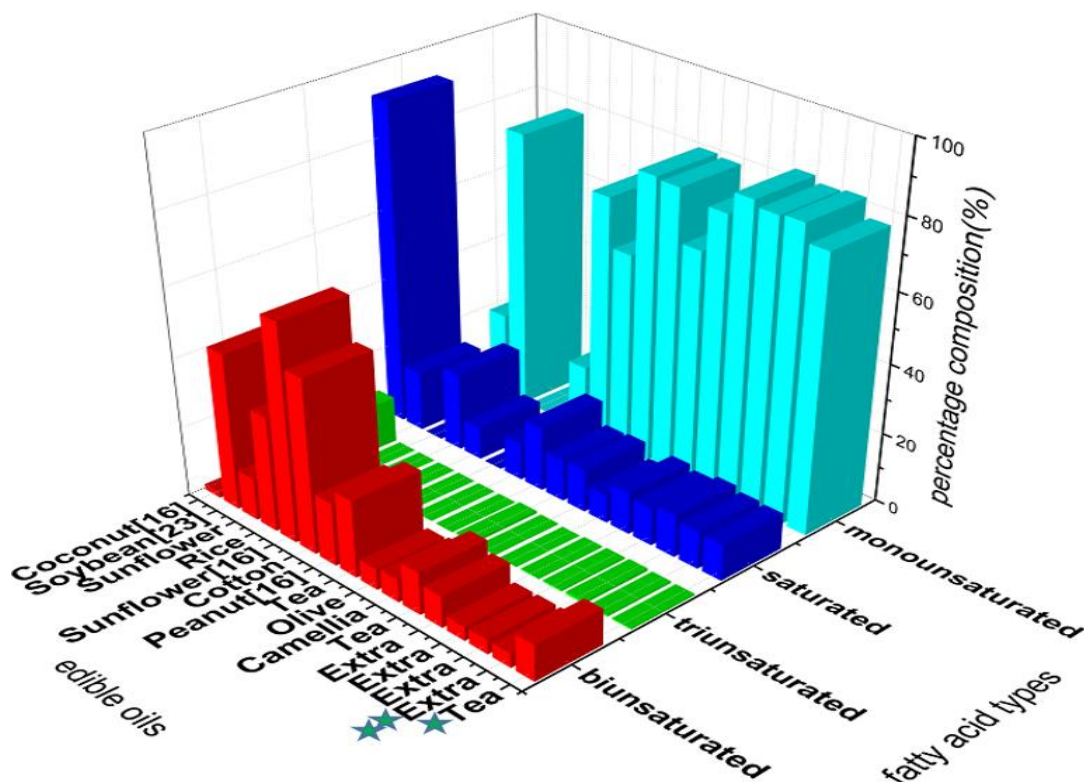


Figure 4: Comparison of levels in (%) of C₁₈ unsaturated and saturated fatty acids in common oils with the present work olive oil and tea oils with star symbols on the chart diagram

Proportions of several acyl groups in different oil samples obtained by ¹H NMR data and classical method of analysis are reported in literature and given in **Figure 4** and **Table S3** [16,17,28,31-35]. It can be seen that the composition of the different oil samples varies

significantly. Guillen *et al.* has analyzed several oil samples and the proportions of acyl groups for extra virgin olive oil is very similar to tea oil and olive oil obtained in the present work. In general, tea oil and extra virgin olive oil samples have the highest proportions of oleic acyl groups and the smallest proportions of linoleic acyl groups compared to other oil samples. Proportions of acyl groups in tea and olive oil samples analyzed in present work are in good agreement with those reported and obtained by ^1H NMR data and classical method of analysis [28,31-35]. The value calculated in our modified method and using the literature mentioned equation is very similar proofing the validity of the simple calculations used in here.

^{13}C NMR analysis

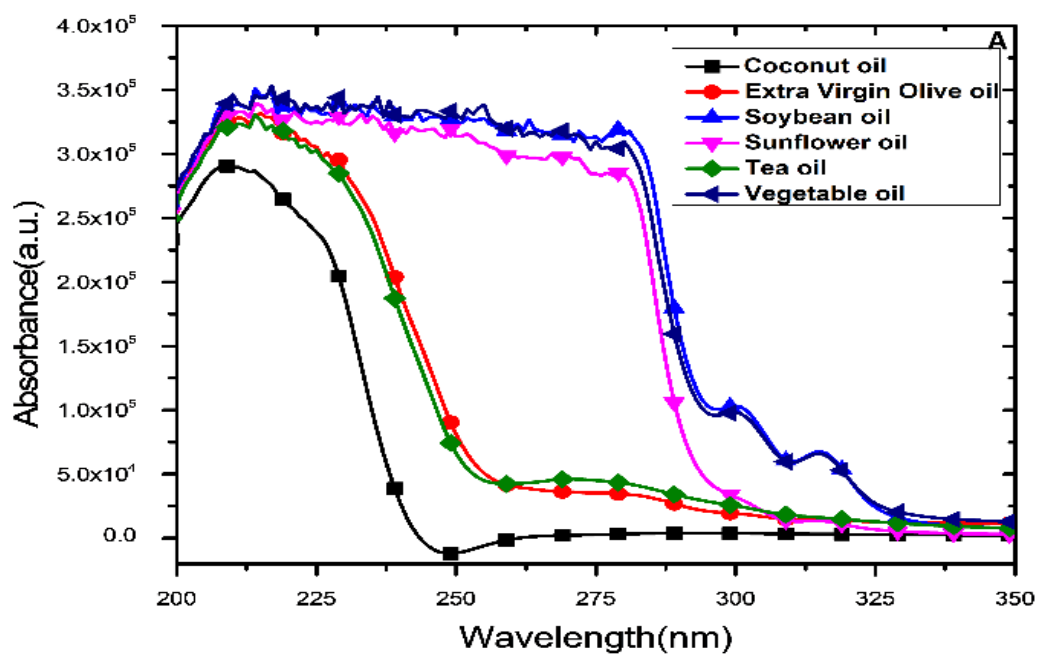
The assignments of carbon resonances of the glycerol moiety and fatty acids are also crucial for the analytical characterization of TAG oils. The ^{13}C NMR spectrum of tea oil was analysed, as this technique has been widely used to study the composition of various oils for both qualitative and quantitative purposes [18]. The ^{13}C NMR spectra is depicted in **Figure S1**. Two sets of signals at δ_{C} 173.1915 and 172.7789 corresponding to the carbonyl carbons (the first carbon in the acyl chains with the higher chemical shift) of the fatty acids on the triglyceride back bone, the higher frequency set at δ_{C} 173.1915 includes the fatty acid chains esterified at α,α -glycerol positions whereas, the low frequency set δ_{C} 172.7789 includes the β -glycerol position chain.

Here, the signals from carbonyl carbons of α,α -chains are shifted about 0.4126 ppm at higher frequency from those of carbonyls of β -chains. This shifting difference was explained by Howarth *et al.*, in 1995, as the carbonyl groups of the β -position chains

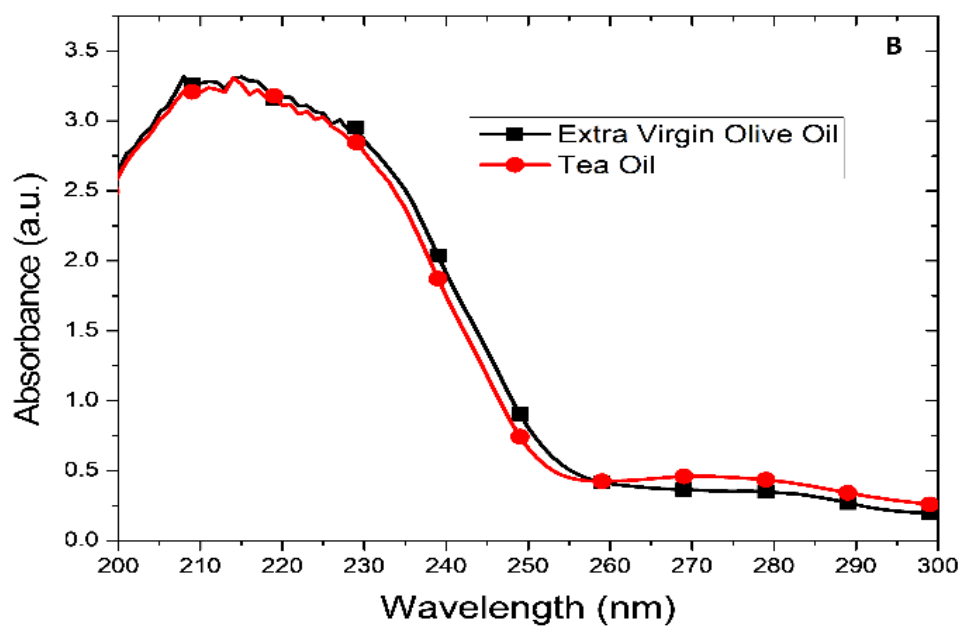
suffer two g-gauche interactions against one interaction intervening for carbonyls of α,α' -chains [12]. Furthermore, the presence of only two carbon signals at δC 62.0638 and 68.8707 with the ratio of 2:1, respectively, correspond to α,α' - and β -positions of the glycerol back bone, confirmed the presence of symmetrical acylglycerols. The 2D-COSY spectrum of tea oil triglycerides (TAG) (**Figure S2**) clearly shows the correlations between the ^1H protons at the main regions like α -hydrogen to the carbonyl carbon, β -hydrogen to the carbonyl carbon, vinyl hydrogen and the tertiary hydrogen Hgl of the glycerine moiety around 5.22 ppm along with the bisallylic hydrogen at a chemical shift of 2.8 ppm.

UV-Visible absorption analysis

The study of UV-Visible absorption spectra of six different oil samples was recorded and the absorbance's were measured at wavelengths range of 200-600 nm (**Figure 5**). The oil samples are coconut oil, olive oil, soybean oil, sunflower oil, tea oil and vegetable oil, respectively. When comparing the maximum absorbance and the area of spectral absorbance from 200 up to 600 nm, the order is vegetable oil > soybean oil > sunflower oil > olive oil > tea oil > coconut oil. The area of absorbance value observed for vegetable oil sample is the highest one while coconut oil exhibited a lower absorbance and was characterized as the good quality oil. The absorption spectra of tea oil were found very similar in pattern and intensity to that of the high-quality extra virgin olive oil for 1% (V/V) samples in hexane (**Figure 5B**). Both tea and extra virgin olive oil have the next lowest absorbance next to the high-quality coconut oil.



A



B

Figure 5: A) UV-Visible spectrum of six oil samples B) UV-Visible spectrum of tea and extra virgin olive oil

Earlier, some researchers made use of UV-Visible absorption spectrometry to classify the quality of olive oils, and the best olive oil quality was identified by the highest absorbance at 212 nm, whilst the worst quality olive oil exhibited by the lowest absorbance in this wavelength [20]. Following this approach, we analyzed tea oil together with five others, including coconut, extra virgin olive, soybean, sunflower and vegetable oils. As shown in **Figure 5A**, significant difference was observed as comparing tea oil with coconut, soybean, sunflower and vegetable oils, and no substantial difference in between the tea oil and extra virgin olive oil. Given this, we claim that the tea oil has a high quality comparable to that of extra virgin olive oil.

Conclusion

We have quantified and compared the TAG profiles of Taiwan tea oil and extra virgin olive oil using high-resolution ^1H NMR (800 MHz) and UV–Visible spectroscopy. The TAG composition of tea oil was determined as 74.83% oleic (ω -9), 10.14% linoleic (ω -6), 0.38% linolenic (ω -3), and 14.65% saturated fatty acids. Oleic acid (ω -9) was the major component. For extra virgin olive oil, the corresponding values were 81.06% oleic, 4.14% linoleic, 0.26% linolenic, and 14.54% saturated fatty acids. Olive oil is well known for its health benefits, including lowering LDL cholesterol, reducing cardiovascular disease risk, and enhancing the absorption of vitamins A, D, E, and K—effects largely attributed to its high monounsaturated fatty acid content. The comparable monounsaturated content observed in tea oil, consistent with reported literature, suggests similar potential health benefits. Overall, the TAG profiles of tea seed oil and extra virgin

olive oil are highly similar according to both our results and previously reported studies. The UV–Visible absorption spectra measured for six edible oils further support this observation: the spectral pattern and intensity of tea oil closely match those of high-quality extra virgin olive oil in 1% (v/v) hexane solutions.

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Conflict of Interest

We wish to assure that our manuscript does not contain any potential interest or "no conflict of interest".

Data Availability Statement

All the data are included in main text and Electronic Supplementary Information (ESI).

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